

## ORIGINAL ARTICLE

**Going ballistic in the plankton: Anisotropic swimming behavior of marine protists**

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**Abstract**

*Diel vertical migrations (DVMs) of many plankton species, including single-celled protists, are well documented in the field and form a core component of many large-scale numerical models of plankton transport and ecology. However, the sparse quantitative data available describing motility behaviors of individual protists have frequently indicated that motility exhibits only short-term correlation on the order of a few seconds or hundreds of micrometers, resembling diffusive transport at larger scales—a result incompatible with DVM, which requires ballistic (straight-line) motion. We interrogated an extensive set of three-dimensional protistan movement trajectories in an effort to identify spatial and temporal correlation scales. Whereas the horizontal components of movement were diffusive, the vertical component remained highly correlated (i.e., nonrandom) for nearly all species for the duration of observation (up to 120 s and 6.1 mm) and in the absence of any environmental cues besides gravity. These persistent motility patterns may have been obscured in some previous studies due to the use of restrictive containers, dimensionally lumped, isotropic analyses, and/or an observation bias, inherent to observing free-swimming organisms with stationary cameras, which we accounted for in this study. Extrapolated over a 12-h period, conservative estimates of vertical travel ranges for the protists observed here would be 3–10 m, while diffusive horizontal motion would result in about 10 cm of travel at most. Hence, these extended observations of phylogenetically diverse swimming protists, coupled with a quantitative analysis that accounts for anisotropy in the data, illustrate the small-scale mechanistic underpinnings of DVM.*

Keywords: plankton, diffusion, motility, correlation, migration

**Introduction**

[1] Plankton are key agents of global biogeochemical cycles (Sherr and Sherr 2002; Strom 2008) and their ecology is in part driven by their motility patterns, which dictate their distributions and encounters with biotic and abiotic targets (Visser and Kiorboe 2006). Motility patterns of many organisms, including plankton, are frequently quantified through random walk models (Codling et al. 2008).

In general, the path of a planktonic organism moving continuously and randomly may appear tortuous at a large scale but nonetheless straight at a sufficiently small scale. Movement along an effectively straight line, where net displacement is proportional to time, is termed “ballistic” (Codling et al. 2008). The scales at which the transition from ballistic to diffusive motility occur are frequently referred to as the correlation length scale  $\lambda$  and time scale  $\tau$ .

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Visser and Kiorboe (2006), inspired by the work of G. I. Taylor (1922) on the dispersal of inert particles in turbulence, presented a method to determine  $\lambda$  and  $\tau$  through analysis of an organisms' root mean square displacement (RMSD) over time. In their laboratory study, Visser and Kiorboe (2006), as well as other researchers employing quantitative analyses of laboratory data (Leptos et al. 2009; Garcia et al. 2011), observed that highly correlated, ballistic movements among protists (e.g., dinoflagellates, ciliates) persisted only for short scales on the order of a few seconds or hundreds of micrometers at most. Short correlation scales imply that cell motility can be modeled with effective diffusivities, or a single isotropic diffusivity in the simplest possible case (Okubo and Levin 2002). Diffusion results in undirected movement and is slow at transporting matter over large scales.

[2] Diffusive motility patterns pose a conundrum. Many planktonic organisms, including both zooplankton and phytoplankton, are known to undergo diel vertical migrations (DVMs) up to tens of meters per day in the field (Hasle 1954; Eppley et al. 1968; Smayda 2010b). Similar but smaller-scale population movements have also been observed in laboratory studies (Eppley et al. 1968; Kamykowski et al. 1998b; Jephson and Carlsson 2009). DVM may allow individuals to avoid predation (Bollens et al. 2012) and, in the case of phytoplankton, balance daytime near-surface light exposure with nighttime nutrient uptake at depth (Cullen 1985).

[3] Although the adaptive advantage of DVM remains unknown, purely diffusive motility patterns lack the directional persistence necessary to support long-range migratory behaviors. DVM can be explained only if essentially ballistic movement patterns persist over very large spatiotemporal scales. In addition to being crucial for DVM, directed vertical swimming may also influence the formation and maintenance of patches and aggregations of plankton (Birch et al. 2009; Steinbuck et al. 2009), effectively counter upwelling currents (Smayda 2010b), and increase encounter rates between organisms (Visser and Kiorboe 2006).

[4] The central role of vertically biased, migratory behaviors in the swimming patterns of protists such as dinoflagellates has been known for some time (Kamykowski 1995). In numerical models of oceanic

plankton populations, the inconsistency of simple diffusion models with DVM has been ameliorated by widespread use of Lagrangian modeling techniques (Broekhuizen 1999; Woods 2005; Yamazaki et al. in press). Briefly, Lagrangian methods allow individual cells or, more tractably, groups of similar individuals to be explicitly accounted for (e.g., assigned vertically oriented swimming velocities) instead of being modeled as undirected, diffusing particles. Although these numerical models assign ballistic motility to plankton, direct empirical demonstration of this swimming behavior is largely missing. There have been a few reports of longer correlation scales in protist swimming paths (Bearon et al. 2004; Jakobsen et al. 2005; Menden-Deuer 2010), as well as qualitative evidence of migratory trajectories in the lab (Kamykowski et al. 1992; Sheng et al. 2010), but quantitative, individual-scale data illustrating ballistic motility are scarce for protists, especially heterotrophic species.

[5] Previous observations of individual plankton motility behaviors were often performed using microscope preparations or other restrictive environments, resulting in spatially, temporally, and dimensionally limited observations and possibly masking natural anisotropic swimming patterns (with the exception of studies noted above). Problems inherent to two-dimensional (2D) and undersampled observations of swimming paths are briefly discussed in Boakes et al. (2011). Fortunately, many analytical and methodological advances have been made since earlier work. Here, we help bridge the experimental gap between individual-level, microscopic behaviors and resultant, larger-scale macroscopic population redistributions by quantifying an extensive data set of three-dimensional (3D) motility patterns for freely swimming protists. We generalize the approach of Visser and Kiorboe (2006), identifying the correlation scales  $\tau$  and  $\lambda$  for each dimension of 3D space independently, in an effort to quantify directional biases in motility behavior. Although ambient water motion, and particularly turbulence, is neglected here for simplicity, as in most previous experimental studies of protist motility, we note that the interplay between ambient currents and swimming orientation (Kessler et al. 1998; Karp-Boss et al. 2000; Durham et al. 2009) is likely to be crucial in the marine environment.

## Methods

### Cell Cultures

[6] We observed a phylogenetically diverse group of free-swimming marine protists in laboratory experiments (Table 1). These include several genera of autotrophic and heterotrophic species.

[7] All phytoplankton cultures were grown in 0.2- $\mu\text{m}$  sterile-filtered autoclaved seawater (FSW), enriched with f/2 nutrients (Guillard 1975). The heterotrophic species (*Favella* sp., *Oxyrrhis marina*, *Protoperdinium bipes*) were cultured in FSW only. All cultures were maintained on a 12:12-h light:dark cycle, at 15 °C, a salinity of 30, and a light intensity of 70–80  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  for the phytoplankton cultures and 8–15  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  for the heterotrophs. The cultures were not axenic. Phytoplankton cultures were transferred every 4–7 d to maintain exponential growth. *Favella* sp. cultures were fed *Heterocapsa triquetra* (final concentration of 200 cells  $\text{mL}^{-1}$ ) and *O. marina* and *P. bipes* were fed *Isochrysis galbana* (final concentration of 1500 cells  $\text{mL}^{-1}$ ) twice a week. Cell concentrations were determined by microscope counts from samples fixed in 1% acid Lugol's solution.

[8] In light of recent evidence, we note that many of the species listed here as autotrophic may be capable of mixotrophy (Stoecker 1999; Jeong et al. 2005; Burkholder et al. 2008). Nonetheless, because the degree of heterotrophy typically appears to be quite minor and we cultured these cells as autotrophs, we refer to them as “autotrophic.”

### Experimental Setup

[9] To quantify movement behaviors, a 30-cm-tall, 5.5-cm-wide, 1-L octagonal acrylic observational chamber was used, as described in Menden-Deuer and Grünbaum (2006). This chamber is orders of magnitude larger than the vessels used in previous studies (Kamykowski et al. 1992; Jakobsen et al. 2005; Sheng et al. 2010; but see Bearon et al. 2004). The tank was located in a dark room to eliminate light responses and filled with FSW by using a peristaltic pump; this method suppressed fluid convection in the chamber (Bearon et al. 2006). The same source water was used for all experiments and cultures. Using a syringe, organisms were introduced at the bottom of the tank through 1-mm internal diameter silicone tubing. Cells were introduced slowly at a rate of 10  $\text{mL min}^{-1}$  to reduce stress to cells, as well as disturbance to the water column. Average final concentrations during filming were typically on the order of 100 cells  $\text{mL}^{-1}$  (Table 1).

[10] Two infrared-sensitive cameras (Pixelink) with Nikon 60-mm Micro Nikkor lenses each monitored a 2D field of view in the horizontal center of the tank, enabling reconstruction of 3D movement behaviors within a 1.5 cm  $\times$  3.3 cm  $\times$  1.3 cm (6.4 mL) observation volume. Cells in the observation volume were at least 500 body lengths from the nearest wall, but because protists live in a low Reynolds number regime where effects of nearby surfaces can be nonintuitively large (White 1946; Vogel 1994), we cannot definitively rule out wall effects. Organisms were illuminated with

**Table 1** Characteristics of species included in this study. All species are dinoflagellates except for the tintinnid ciliate *Favella* sp. and the raphidophyte *Heterosigma akashiwo*. Swimming speed is an average of all instantaneous values recorded. Concentrations were computed by first calculating total number of cells added to each replicate observation tank, dividing these by tank volume, and then taking a weighted average across replicates, weighted by number of tracks analyzed. Data are means  $\pm$  standard errors.

Species	Diameter ( $\mu\text{m}$ )	Swimming speed ( $\mu\text{m s}^{-1}$ )	Trophic mode	Concentration (cells $\text{mL}^{-1}$ )	No. tracks
<i>Akashiwo sanguinea</i>	37 $\pm$ 0.7	170 $\pm$ 0.4	Autotrophic	210	34227
<i>Favella</i> sp.	100 $\pm$ 5	750 $\pm$ 3	Heterotrophic	50	11270
<i>Heterocapsa triquetra</i>	17 $\pm$ 0.1	130 $\pm$ 1	Autotrophic	130	612
<i>Heterosigma akashiwo</i>	11 $\pm$ 0.2	150 $\pm$ 0.6	Autotrophic	540	1836
<i>Oxyrrhis marina</i>	22 $\pm$ 0.2	360 $\pm$ 0.9	Heterotrophic	100	4941
<i>Prorocentrum concavum</i>	34 $\pm$ 0.3	220 $\pm$ 1	Autotrophic	240	1581
<i>Prorocentrum micans</i>	35 $\pm$ 0.3	230 $\pm$ 2	Autotrophic	130	476
<i>Protoperdinium bipes</i>	15 $\pm$ 0.2	260 $\pm$ 1	Heterotrophic	560	2800
<i>Scrippsella trochoidea</i>	22 $\pm$ 0.2	200 $\pm$ 5	Autotrophic	130	42

infrared (960 nm) light-emitting diodes to eliminate the potential for light-mediated behavioral responses. Video was captured at 15 frames s<sup>-1</sup>. Over the course of each experiment, which lasted several hours, the cameras repeatedly traversed several vertical horizons, filming at each location for 1–3 min, depending on species. Since we were unable to keep track of individual cell identities between video segments, it is conceivable that some individuals were represented by multiple tracks (e.g., they swam up through the field of view and later swam back down).

[11] 3D swimming paths were determined by first assembling 2D trajectories from Cartesian coordinates of each organism in each stereoframe and then joining 2D tracks based on matching space–time occurrence in the two 2D segments. To reduce spurious noise, the 3D paths were smoothed using cubic splines in each dimension and resampled at the filming rate (every 0.07 s) for subsequent RMSD analysis (for more details, see Menden-Deuer and Grünbaum 2006; Harvey and Menden-Deuer 2011).

[12] Postprocessing of the 3D paths was performed to remove spurious trajectories and paths not belonging to live organisms. Filtering metrics included a minimum track duration (5 s), minimum arc length (1 mm), minimum coefficient of determination  $R^2$  for the fitted splines in each dimension (0.99), and maximum median path curvature (0.1  $\mu\text{m}^{-1}$ ). In addition, if visual inspection of the paths from a video segment indicated sweeping, arc-like trajectories indicative of fluid convection, all paths from that video segment were discarded. In total, we acquired about 58,000 swimming tracks (Table 1) that were 16 s long on average and up to 120 s long.

### Data Analysis

[13] Following Visser and Kiorboe (2006), the correlation time scale  $\tau$  and length scale  $\lambda$  were estimated by curve fits of the data to Taylor's equation (Taylor 1922), which describes the transition from ballistic to diffusive motion of a particle undergoing a continuous random walk:

$$l(t) = [2\nu^2\tau(t - \tau(1 - e^{t/\tau}))]^{1/2}, \quad (1)$$

where  $l$  is the expected net displacement of the particle (or organism) after time  $t$ . The equation has two parameters:  $\tau$  and the effective movement speed,  $\nu$ . Note that  $\lambda = \nu\tau$ .

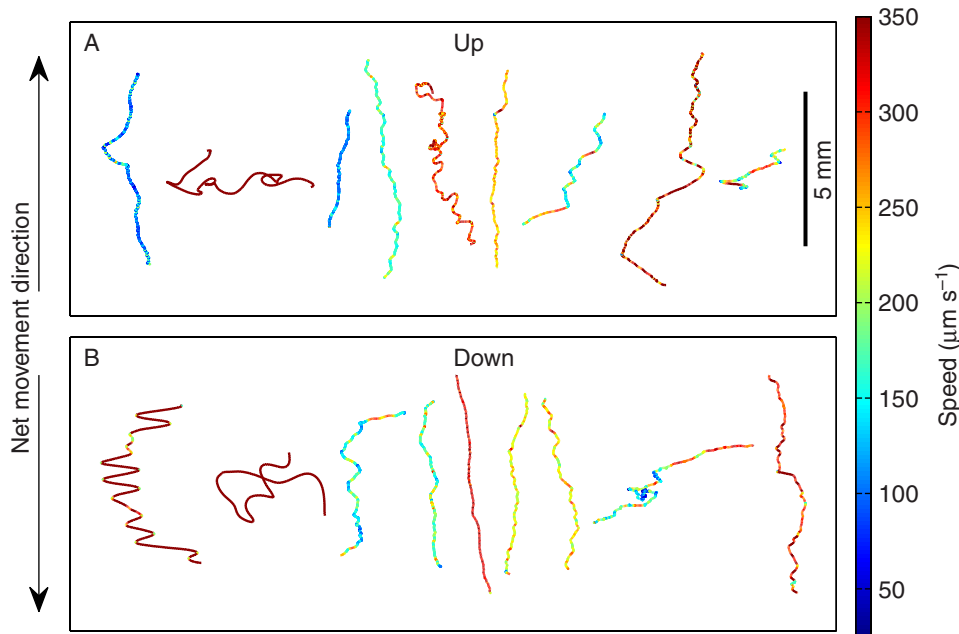
[14] For a large group of organisms,  $l(t)$  can be equated to the RMSD from the (arbitrary) starting point of each recorded path, where the mean is an ensemble average across individuals (Berg 1993). Since a preliminary inspection of the observed movement trajectories suggested a strong vertical bias in motility for most species (Fig. 1), we computed RMSD for the vertical dimension separately from RMSD in the horizontal plane:

$$\text{RMSD}_z(t) = \left[ \frac{1}{n} \sum_{i=1}^n \{z(i, t) - z(i, 0)\}^2 \right]^{1/2}, \quad (2)$$

$$\begin{aligned} \text{RMSD}_{xy}(t) \\ = \left[ \frac{1}{n} \sum_{i=1}^n \{x(i, t) - x(i, 0)\}^2 + \{y(i, t) - y(i, 0)\}^2 \right]^{1/2}, \end{aligned} \quad (3)$$

where  $n$  is the number of tracks analyzed,  $[x(i, t), y(i, t), z(i, t)]$  is the position of organism  $i$  at time  $t$ , and  $[x(i, 0), y(i, 0), z(i, 0)]$  is its initial position. Equating each of these expressions to Eq. 1, the nonlinear curve fitting routine `nlinfit()` from the Statistics Toolbox of MATLAB 7.12.0 was used to estimate  $(\nu_z, \tau_z)$  and  $(\nu_{xy}, \tau_{xy})$  for the vertical and horizontal dimensions, respectively. Thus, this dimensionally decomposed analysis yields separate correlation scales for the vertical and horizontal dimensions to reveal any directional bias in movement.

[15] In experiments utilizing stationary cameras and free-swimming organisms, the observations of greatest duration are more likely to belong to slower-moving individuals. This observation bias leads to an inverse correlation between swimming speed and track duration and can affect the calculation of  $\tau$ , since the swimming speed  $\nu$  is implicitly assumed to be constant over time in Eq. 1. To correct for this observational bias, subsets of the swimming tracks were analyzed separately by grouping tracks of similar duration (and thus average speed) together.



**Fig. 1** Examples of observed net upward-oriented (A) and downward-oriented (B) swimming paths, colored according to instantaneous speed. Higher-frequency details have been smoothed via moving averages for clarity. In both panels, one path per species is shown; from left to right: *Akashiwo sanguinea*, *Favella* sp., *Heterocapsa triquetra*, *Heterosigma akashiwo*, *Oxyrrhis marina*, *Prorocentrum concavum*, *Prorocentrum micans*, *Protoperdinium bipes*, and *Scrippsiella trochoidea*. Each 3D path has been projected onto a vertical plane that maximizes the apparent horizontal distance between starting and ending points. Even with this conservative bias in viewing angle, net vertical displacements are still typically much larger than net horizontal displacements.

[16] Finally, diffusivities that describe the large-scale motion of the organisms were calculated from  $v$  and  $\tau$  when those parameters existed. In the 2D horizontal plane,  $D_{xy} = v_{xy}^2 \tau_{xy} / 2$ , and in the vertical dimension,  $D_z = v_z^2 \tau_z$  (Visser and Thygesen 2003). Note that initially we did further separate the  $x$ - and  $y$ -directions from each other, but as expected, differences were unremarkable (e.g., all  $D_x$  were of the same order of magnitude as the corresponding  $D_y$ ) due to lack of horizontal gradients.

## Results

### Vertical Swimming Bias

[17] An inspection of some representative swimming paths (Fig. 1) revealed a vertical bias in the trajectories of both upward- and downward-swimming cells of most species. This bias may generally be stronger in autotrophs; the tracks of the heterotrophs *Protoperdinium bipes* and *Favella* sp., but not *Oxyrrhis marina*, appear less vertically oriented than tracks of the

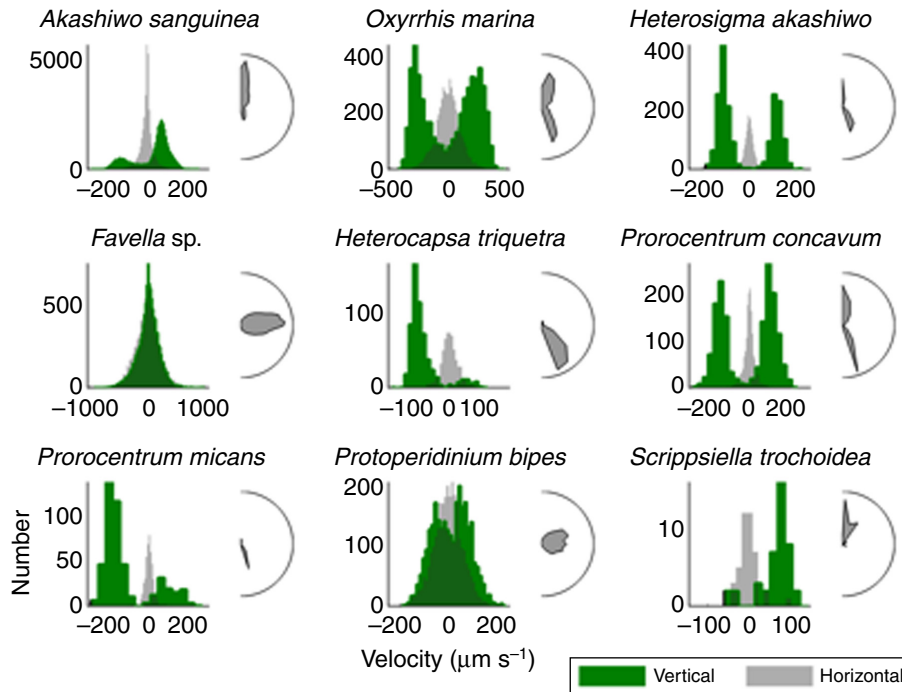
autotrophic species. Nonetheless, vertically biased swimming behavior was typical overall.

[18] The vertical bias suggested by the tracks in Fig. 1 is consistent with histograms of average vertical velocity along each path (Fig. 2). This vertical bias is also evident in polar histograms of the average declination angle of each track (Fig. 2). Many species, such as *Heterosigma akashiwo* and *Prorocentrum concavum*, exhibited bimodal distributions consisting of “up-swimmers” and “down-swimmers,” with most cells moving within  $45^\circ$  of vertically up or down. The ciliate *Favella*

sp. was the primary exception, though we note that vertical migrations have been reported for other ciliate genera (Dale 1987; Jonsson 1989; Pérez et al. 2000). Since there were no gradients in the horizontal plane during our experiments (e.g., of light or nutrients), histograms of the horizontal velocity components were expectedly unimodal around zero for all species (Fig. 2).

[19] The fraction of upward- versus downward-oriented paths and the  $p$ -values resulting from Hartigan’s dip test (Hartigan and Hartigan 1985) of unimodality (Table 2) indicate with high probability that more than one mode (i.e., bimodality) was present for vertical velocity in six of the nine species examined. The standard errors in the upward or downward fraction (Table 2), computed using each filming horizon (i.e., vertical location) and time interval as a statistical observation, indicate little variation throughout the tank or over the duration of each experiment for most species. Hence, distinct up-swimming and down-swimming groups usually co-occurred at all times and locales





**Fig. 2** Histograms of average vertical velocity (green) and average x-component of horizontal velocity (gray) along with polar histograms of the declination angle of each track from vertical. Whereas the horizontal velocity was always unimodal and centered at zero, a bimodal distribution was frequently evident in vertical velocity. There was also a strong vertical bias in swimming direction. *Favella* sp. did not follow this pattern, exhibiting neither bimodality nor vertical bias.

In addition, a steady state of nearly 50% up-swimmers and 50% down-swimmers was sometimes but not always observed, suggesting that cells did not always simply reverse direction upon encountering the water surface or tank bottom. An inherent 50/50 split in vertical swimming direction has been termed “neutral gravitaxis” but seems to be an exceedingly rare observation (Machemer-Röhnisch et al. 1993). Regardless of whether the protists observed ever turned around outside the cameras’ field of view, any cells in the marine environment undergoing DVM would need to alternate direction eventually, on a time scale longer than the experiments.

[20] Mean downward velocities were greater than

within theoretically clonal populations. However, no clear generalization can be made regarding the proportion of up-swimmers versus down-swimmers across species (e.g., between autotrophs and heterotrophs).

mean upward velocities (paired  $t$ -test,  $t_8 = -3.3$ ,  $p = 0.01$ ; Table 2), most likely due to the addition of passive sinking speed to the former, as observed by Kamykowski et al. (1992) for several dinoflagellate

**Table 2** Parameters describing the vertical velocity distributions of all species, listed for up- and down-swimming groups,  $\pm$  standard errors where appropriate. Unimodality was rejected when Hartigan’s  $p$ -value  $< 0.05$ . See number of tracks listed in Table 1 for degrees of freedom in Hartigan’s tests.

Species	Fraction of total tracks		Hartigan’s dip test		Mean vertical velocity ( $\mu\text{m s}^{-1}$ )		Maximum vertical velocity ( $\mu\text{m s}^{-1}$ )	
	Up	Down	$p$ -Value	Dip statistic	Up	Down	Up	Down
<i>Akashiwo sanguinea</i>	$0.71 \pm 0.02$	$0.29 \pm 0.02$	$< 0.0001$	0.019	$73 \pm 0.2$	$-109 \pm 0.5$	249	-285
<i>Favella</i> sp.	$0.54 \pm 0.009$	$0.46 \pm 0.009$	$> 0.99$	0.0014	$137 \pm 2$	$-152 \pm 2$	985	-940
<i>Heterocapsa triquetra</i>	$0.10 \pm 0.06$	$0.90 \pm 0.06$	0.66	0.014	$54 \pm 3$	$-94 \pm 1$	117	-179
<i>Heterosigma akashiwo</i>	$0.41 \pm 0.05$	$0.59 \pm 0.05$	$< 0.0001$	0.11	$116 \pm 1$	$-111 \pm 0.8$	225	-189
<i>Oxyrrhis marina</i>	$0.54 \pm 0.02$	$0.46 \pm 0.02$	$< 0.0001$	0.068	$208 \pm 2$	$-236 \pm 2$	460	-436
<i>Prorocentrum concavum</i>	$0.54 \pm 0.02$	$0.46 \pm 0.02$	$< 0.0001$	0.11	$113 \pm 1$	$-157 \pm 2$	238	-308
<i>Prorocentrum micans</i>	$0.22 \pm 0.07$	$0.78 \pm 0.07$	$< 0.0001$	0.041	$113 \pm 6$	$-185 \pm 2$	265	-306
<i>Protopteridinium bipes</i>	$0.54 \pm 0.02$	$0.46 \pm 0.02$	0.014	0.012	$66 \pm 1$	$-59 \pm 1$	223	-194
<i>Scrippsiella trochoidea</i>	$0.70 \pm 0.1$	$0.30 \pm 0.1$	0.95	0.038	$73 \pm 4$	$-159 \pm 6$	170	-260

species. However, this generalization does not hold for maximum velocities ( $t_8 = -1.1$ ,  $p = 0.32$ ): four of the nine species (including all three heterotrophic species) appear to occasionally expend additional effort when swimming upward. Maximum vertical velocities were approximately twice as large as mean vertical velocities for both up- and down-swimmers; the differences would be much larger if instantaneous maxima were used, but we believe maxima of track averages (where analyzed tracks were at least 1 mm long or 10–100 body lengths) are more meaningful in most ecological contexts, such as large-scale migratory behaviors. If these speeds were maintained along the same heading for 12 h, travel ranges on the order of meters (3–10 m based on mean velocities, 6–19 m based on maximum velocities) could be achieved.

#### Horizontally Diffusive Movement

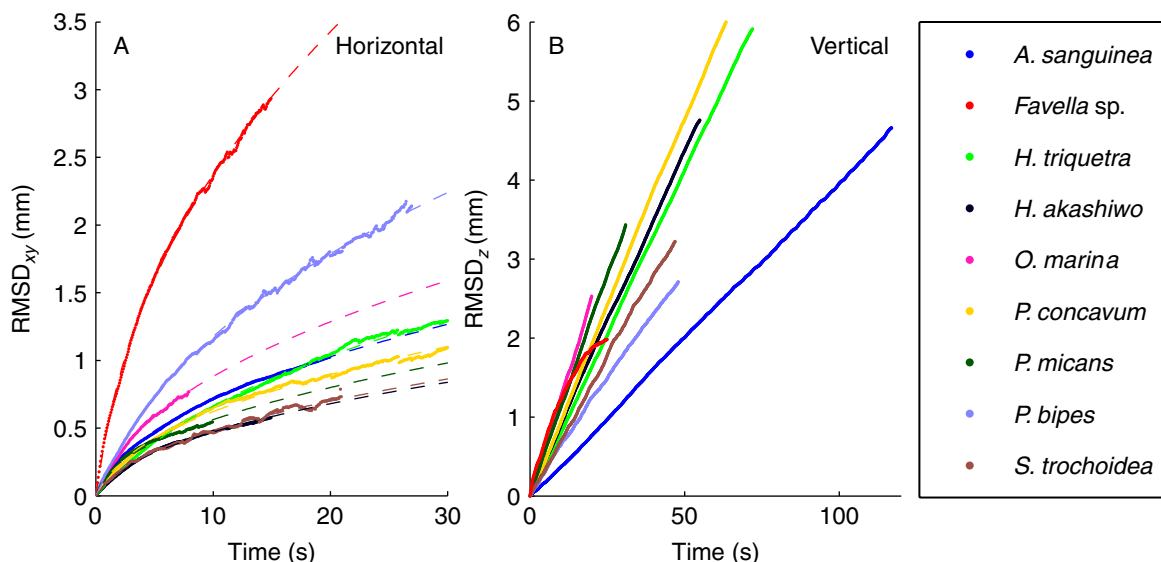
[21] Decorrelation of horizontal swimming direction is apparent in the nonlinearity of the  $\text{RSMD}_{xy}(t)$  curves (Fig. 3A), and Taylor's equation provides a good fit to the data. Estimates of the horizontal decorrelation time  $\tau_{xy}$  (Table 3) ranged from fractions of a second to a few seconds, and horizontal diffusivity values  $D_{xy}$  were on the order of  $10^{-8} \text{ m}^2 \text{ s}^{-1}$  (except  $10^{-7} \text{ m}^2 \text{ s}^{-1}$  for fast-

moving *Favella* sp.). Thus, at time scales longer than a few seconds and spatial scales larger than 1 mm, these protists dispersed at the relatively slow rate of diffusive motion in the horizontal plane.

[22] Because of the typical separation of organisms into up-swimmers and down-swimmers, we also analyzed horizontal movements separately for up- and down-swimmers to check for a correlation between this vertical dichotomy and horizontal movement. However, the horizontal movement parameters for each group were similar (e.g.,  $D_{xy}$  always within a factor of 3 between separate vs. combined analyses). Thus, irrespective of whether cells swam up or down, their horizontally diffusive motility behaviors were similar.

#### Vertically Ballistic Movement

[23] When  $\text{RSMD}_z$  is plotted versus time for each species (Fig. 3B), the result is a straight line in every case except *Favella* sp. (which transitioned to diffusive motility within a few seconds). Nearly all the species studied exhibited ballistic behavior in the vertical dimension, and a lack of decorrelation of vertical direction (up vs. down) for the extent of the observed trajectories is evident—up to 120 s or 6 mm in some cases (Table 3).



**Fig. 3** A — Horizontal root-mean-square displacement,  $\text{RSMD}_{xy}(t)$ , versus time for each species. All species fully transitioned to horizontally diffusive motion within  $\sim 5$  s or  $650 \mu\text{m}$ ; extrapolated curve fits to Taylor's equation are shown with dashed lines. B — Vertical root-mean-square displacement,  $\text{RSMD}_z$ , versus time for each species. Almost all species exhibited vertically ballistic movement for the duration of the observations. The fast-swimming *Favella* sp. was an exception, moving diffusively both horizontally and vertically. Note the different y-axis ranges in A and B.

**Table 3** Motility parameters (i.e., effective swimming speed  $\nu$ , correlation time scale  $\tau$ , correlation length scale  $\lambda$ , and effective diffusivity  $D$ ) for the horizontal ( $xy$ ) and vertical ( $z$ ) components of movement. For the horizontal case, parameters  $\pm$  standard errors were derived from nonlinear fits of horizontal root-mean-square displacement,  $\text{RSMD}_{xy}(t)$ , to Eq. 1. For the vertical case, minimum possible bounds on  $\tau_z$  and  $\lambda_z$  were estimated based on the extents of the linear (i.e., ballistic)  $\text{RSMD}_z(t)$  data plotted in Fig. 3B. However, values for *Favella* sp. are exact since decorrelation of  $\text{RSMD}_z(t)$  was observed in this species.

Species	Horizontal				Vertical	
	$\nu_{xy}$ ( $\mu\text{m s}^{-1}$ )	$\tau_{xy}$ (s)	$\lambda_{xy}$ ( $\mu\text{m}$ )	$D_{xy}$ ( $10^{-8} \text{ m}^2 \text{ s}^{-1}$ )	Minimum $\tau_z$ (s)	Minimum $\lambda_z$ (mm)
<i>Akashiwo sanguinea</i>	200 $\pm$ 0.6	0.80 $\pm$ 0.03	160 $\pm$ 3	1.6 $\pm$ 0.01	120	4.7
<i>Favella</i> sp.	710 $\pm$ 5	0.86 $\pm$ 0.01	610 $\pm$ 6	22 $\pm$ 0.05	2.4	0.48
<i>Heterocapsa triquetra</i>	100 $\pm$ 0.4	4.0 $\pm$ 0.05	400 $\pm$ 3	2.0 $\pm$ 0.006	72	5.9
<i>Heterosigma akashiwo</i>	130 $\pm$ 2	0.87 $\pm$ 0.02	110 $\pm$ 2	0.76 $\pm$ 0.003	55	4.8
<i>Oxyrrhis marina</i>	250 $\pm$ 2	1.1 $\pm$ 0.03	280 $\pm$ 5	3.4 $\pm$ 0.03	20	2.5
<i>Prorocentrum concavum</i>	210 $\pm$ 6	0.58 $\pm$ 0.04	120 $\pm$ 4	1.2 $\pm$ 0.004	65	6.1
<i>Prorocentrum micans</i>	300 $\pm$ 20	0.21 $\pm$ 0.02	64 $\pm$ 3	0.98 $\pm$ 0.004	31	3.4
<i>Protoperidinium bipes</i>	210 $\pm$ 1	2.6 $\pm$ 0.04	530 $\pm$ 4	5.5 $\pm$ 0.02	48	2.7
<i>Scrippsiella trochoidea</i>	180 $\pm$ 5	0.45 $\pm$ 0.03	79 $\pm$ 3	0.70 $\pm$ 0.005	47	3.2

### Correction of Observation Bias

[24] Although swimming speed was constant on average along each individual swimming track (no significant trend; two-tailed  $Z$ -test,  $Z_{57783} = 3.4$ ,  $p = 0.77$ ), there was a highly significant inverse correlation between track-averaged speed and track duration (Fig. 4). This caused an apparent deceleration in  $\text{RSMD}_z(t)$  in many species since the pool of tracks used in the population average consisted of longer, slower tracks as time increased (Fig. 4). An observation bias, therefore, caused the motility behaviors to erroneously appear diffusive before any corrective procedures were applied; decreasing average speed due to the observation bias had the same effect as the accumulated turns of diffusive motility. Once the pool of analyzed tracks was instead chosen to contain only paths of a narrow range of durations, the apparent diffusive behavior disappeared (Fig. 4). We examined several choices of track duration range (e.g., 89.9–90th, 99.9–100th percentiles), and although each data subset yielded a differently sloped  $\text{RSMD}_z(t)$  line (i.e., a different average vertical swimming speed), we did not identify decorrelation in the vertical direction for any subset of tracks (except for *Favella* sp.). Therefore, tracks with the longest durations were used to generate Fig. 3B and to obtain the minimum bounds on  $\tau_z$  and  $\lambda_z$  in Table 3. To our knowledge, this type of observation bias has not yet been recognized, even though it may be pervasive. Although the issue might also be expected to affect  $\text{RSMD}_{xy}(t)$ , applying a similar

correction technique in that case yielded nearly identical results since horizontal motility was actually diffusive. Thus, it appears that the bias correction applied here correctly identifies ballistic motion but does not erase a diffusive movement pattern when it exists.

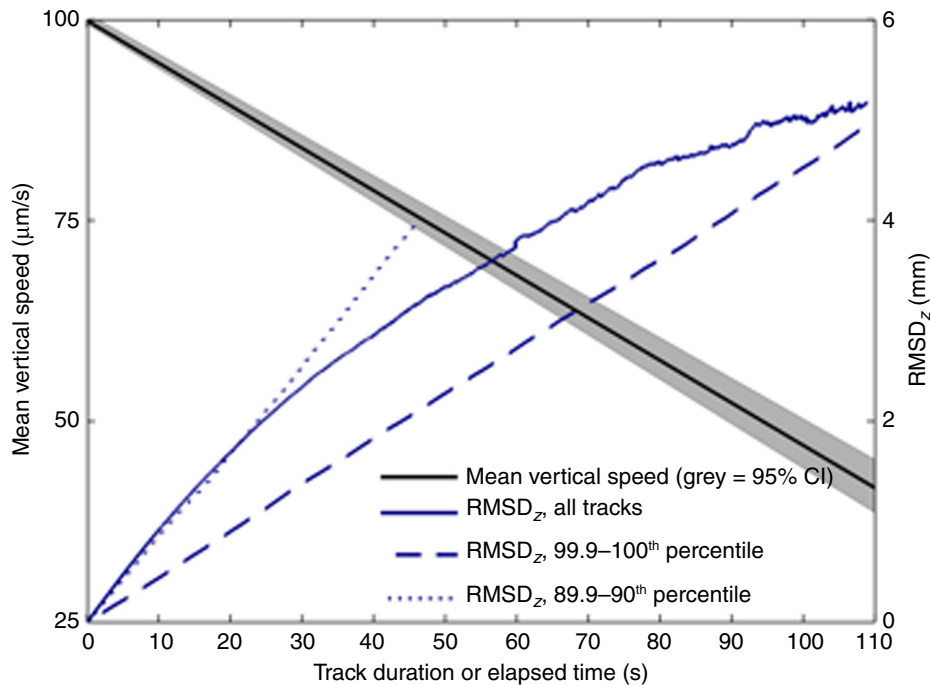
### Discussion

#### Anisotropic Plankton Motility

[25] Here we provide empirical evidence of highly directed movement behaviors for both autotrophic and heterotrophic planktonic protists. These documented vertically biased, nondiffusive motility patterns at the level of individual cells are consistent with and essential to large-scale planktonic vertical migrations that have been observed in the field. Both DVM (reviewed in Smayda 2010b) and vertically biased protistan motility (reviewed in Kamykowski 1995) have been well known for some time. However, there has been a paucity of direct, individual-level observations of these behaviors. The present study addresses this issue and links individual-scale motility behaviors with population distributions in a direct, quantitative sense.

[26] By analyzing the spatial dimensions of protistan swimming separately, we documented considerable vertical bias in their movements that would have been concealed in a simpler lumped analysis. Furthermore, vertical swimming directions exhibited long-range correlation on scales of at least minutes and millimeters. These correlation scales are undoubtedly





**Fig. 4** Track-averaged vertical speed versus track duration and  $\text{RMSD}_z(t)$  for *Akashiwo sanguinea*. An observation bias caused the average speed (i.e., slope of  $\text{RMSD}_z(t)$ ) of shorter tracks to be greater than that of longer tracks. Movement therefore erroneously appeared diffusive if all tracks were used to calculate  $\text{RMSD}_z$ , but if any subset of tracks of similar duration was analyzed, the observation bias was removed,  $\text{RMSD}_z(t)$  remained linear, and movement appeared ballistic.

underestimates, given our finite observation volume and duration. If nonstatic observation methods were used (i.e., cameras translating with the organisms), even longer tracks might be recorded, and the minimum bounds on  $\tau_z$  and  $\lambda_z$  would likely increase further.

[27] If the vertically ballistic swimming patterns observed here were extrapolated over a 12-h period, vertical travel ranges would be on the scale of meters (up to 20 m for *Oxyrrhis marina* swimming at about  $450 \mu\text{m s}^{-1}$ ). In contrast, horizontal swimming would result in travel distances 2 orders of magnitude shorter, on the order of 10 cm, since motility in the horizontal plane was clearly diffusive down to scales of  $<1$  mm. Furthermore, the difference in dispersal is not constant but grows over time due to  $\text{RMSD}(t)$  being proportional to  $t$  for ballistic motion and  $\sqrt{t}$  for diffusion. These conclusions apply to all the species studied except *Favella* sp., which displayed diffusive motility with small  $\tau$  and  $\lambda$  in all dimensions.

[28] The empirical documentation of anisotropic swimming patterns presented here indicates that an

isotropic diffusion model (Okubo and Levin 2002) of population dispersal is likely to be invalid for many planktonic protists, as such a pattern is inconsistent with the swimming behaviors of all species examined here except *Favella* sp. In these experiments, each population consisted of cells with a substantial range of mean swimming speeds, as opposed to all cells having the same average vertical speed with instantaneous random perturbations. The latter scenario might lend itself to an Eulerian advection-diffusion model of vertical dispersal that incorporates a constant drift velocity (Hill and

Häder 1997; Bearon and Grünbaum 2008; Li et al. 2009). Instead, our data suggest that a model of vertical dispersal in which all cells move at unique, steady speeds according to their species' overall probability distribution is most appropriate and is consistent with empirically validated model simulations (Menden-Deuer 2010). This Lagrangian type of approach to modeling plankton movement has been employed in a large number of numerical modeling studies (Broekhuizen et al. 2003; Ross and Sharples 2008; Yamazaki et al. in press) and is validated by the empirical results presented here.

[29] Besides the vertically biased trajectories, another obvious feature of these data is the frequent presence of bimodal vertical velocity distributions (i.e., comparable fractions of up- and down-swimmers), even at the beginning of the experiments. This is intriguing since each experiment consisted of theoretically clonal cell populations from the same culture exposed to only one constant, unidirectional environmental cue: gravity. Opposite responses to light and gravity have previously been reported for different dinoflagellate species

(Kamykowski et al. 1998b) but rarely within subpopulations of the same strain at the same time (but see Machemer-Röhnisch et al. 1993; Bearon et al. 2004). Given the lack of obvious external factors that would differ between these subpopulations, our data support the hypothesis that swimming behavior is driven at least in part by intracellular biochemical status (Kamykowski et al. 1998a; Grünbaum 2000), and this is assumed in some numerical models of protistan vertical migration (Kamykowski and Yamazaki 1997; Broekhuizen et al. 2003; Ralston et al. 2007).

### Previous Studies

[30] Isotropic motility parameters ( $\tau \sim 1$  s and  $D \sim 10^{-8} \text{ m}^2 \text{ s}^{-1}$ ) have been reported for several protist species (Visser and Kiorboe 2006; Leptos et al. 2009; Garcia et al. 2011). These values are similar to the corresponding parameters reported here for the horizontal plane. Prior reports of diffusive movement patterns in all directions may have been due to spatially or temporally restrictive observation conditions compared with the free-swimming protists observed here. We note that Sheng et al. (2010) calculated substantially higher horizontal and vertical diffusivities of  $10^{-7}$  to  $10^{-6} \text{ m}^2 \text{ s}^{-1}$  for both the mixotrophic dinoflagellate *Karlodinium veneficum* and its prey, the cryptophyte alga *Storeatula major*. Nevertheless, diffusivities of this magnitude are still inconsistent with multimeter-scale vertical migrations.

[31] There have been several reports of effectively ballistic motion for extended periods, both in a qualitative sense (Kamykowski et al. 1992; Sheng et al. 2010) and through quantitative measurements of correlation scales: 10 s in *Heterocapsa triquetra* (Jakobsen et al. 2005), about 1 min in some strains of *H. akashiwo* (Bearon et al. 2004), and strongly suggested to be at least several minutes for *Oxyrrhis marina* via model simulations (Menden-Deuer 2010). The data presented here provide additional quantitative, organism-scale evidence of prolonged vertically ballistic swimming in these as well as several other species. Since ballistic movement occurred for the duration of these observations, our estimates of  $\tau_z$  and  $\lambda_z$  are conservative, and their true values are likely to be much greater than the minimum bounds given in Table 3. To increase

confidence in our results, we performed a sensitivity analysis of each set of fitted motility parameters as a function of the extent of data used (i.e., by cutting off the  $\text{RMSD}(t)$  data prematurely and examining the fit parameters as a function of the cutoff time). For  $\text{RMSD}_{xy}(t)$ , after an initial transitory period, a plateau region was always present during which  $v_{xy}$  and  $\tau_{xy}$  remained nearly constant. This indicates that the duration of swimming trajectories was sufficient to accurately quantify diffusive motility in the horizontal plane. In contrast, all  $\text{RMSD}_z(t)$  curves were completely linear, characteristic of ballistic motility, and only minimum estimates of  $\tau_z$  could be determined, except for the ciliate *Favella* sp.

### Diel Vertical Migration

[32] A strong and persistent vertical bias in individual protistan swimming patterns is necessary to facilitate well-documented DVM behaviors. Although direct observations of individual cells over a scale of meters are not currently possible, migratory vertical ranges of 5–75 m (typically 10–20 m) over 12 h have been inferred from population-level field observations from many studies (reviewed in Smayda 2010b). Extrapolating the mean and maximum vertical swimming speeds observed here (Table 2) yields theoretical 12-h ranges of 3–10 m and 6–19 m, respectively. These values may be conservative since instantaneous maximum speeds were higher. Hence, our individual-scale laboratory observations are consistent with large-scale DVM behavior in both qualitative (i.e., ballistic instead of diffusive vertical motion) and quantitative terms (i.e., necessary vertical swimming speeds).

### Encounter Rates

[33] Consequences of ballistic versus diffusive motility in the context of microscopic predator-prey interactions have been explored previously. Encounter rates predicted for organisms moving purely ballistically are higher than for organisms moving purely diffusively (i.e., following a Brownian random walk) through a uniform environment (Visser and Kiorboe 2006) since there is no backtracking of previously covered areas in the former. Although the relative advantage of one movement type over another can be very small under

some circumstances (e.g., densely spaced, fast-moving targets) (Bartumeus et al. 2002; Viswanathan et al. 2002), protists moving ballistically in the vertical dimension would nevertheless be expected to have higher encounter rates than those moving diffusively in all directions. Fitness of protistan searchers in the dilute ocean environment is likely to be enhanced further through nutrient- or prey-induced behavioral changes (e.g., higher turning rates) that can lead to well-documented consumer aggregations within resource patches (Menden-Deuer and Grünbaum 2006; Stocker et al. 2008; Seymour et al. 2009).

[34] Since targets can include both prey and predators, the enhanced encounter rates resulting from highly correlated movements are likely to result in a motility trade-off. Visser and Kiorboe (2006) suggested that an organism's correlation length scale  $\lambda$  is likely to be smaller than the detection radius of its predator but larger than its own resource detection radius, minimizing its risk of predation at little cost to its own foraging efficiency. The large-scale vertically ballistic motion observed here would seem to pose an increased risk from uniformly distributed predators. However, this may be partially mitigated by escape responses or more complex movement patterns than those discussed here (Visser 2007; Harvey and Menden-Deuer 2012). Alternatively, since nutrients and prey are often patchily distributed (Mitchell et al. 2008; Durham and Stocker 2012), the increased probability of finding unexploited resource patches may simply outweigh the increased mortality risk due to predator encounters. Other possible benefits to ballistic, dispersive swimming behavior include reduced competition among cells (Hamilton and May 1977), reduced risk from predators attracted to high cell concentrations (Tiselius 1992), and reduced risk of the entire population being subjected to a localized risk or condition. The tendency of cluster formation due to rapid asexual reproduction in planktonic organisms (Young et al. 2001) may also be lessened.

#### *Implications for Other Mechanisms*

[35] Although the vast majority of the cells observed moved ballistically in the vertical dimension for as long as we could track them, individuals in nature clearly must be capable of other swimming strategies if

DVM is to occur, as well as other phenomena such as plankton layer aggregations that can persist for hours to weeks (Dekshenieks et al. 2001; Menden-Deuer 2008; Cheriton et al. 2009). In models of the latter, cells are sometimes assumed to swim toward a certain depth (Steinbuck et al. 2009). Station-keeping might generally be accomplished by decreasing the ballistic vertical swimming speed, or by turning more frequently and thus switching to a diffusive movement regime. Cues such as varying nutrient concentration and light intensity (MacIntyre et al. 1997; Hall and Paerl 2011; see Kamykowski 1995 for other cues) in the marine environment, which were not present in our experiments, are probably crucial in modulating behavioral switches and are sometimes assumed to determine instantaneous vertical swimming speed and direction in numerical models (Liu et al. 2001; Ross and Sharples 2007, 2008). Resource-induced behavioral switches from vertically ballistic to diffusive motion have also been observed experimentally when herbivorous protists were exposed to patches of phytoplankton prey (Menden-Deuer and Grünbaum 2006), with qualitatively similar results reported for fish larvae and nauplii prey (Mahjoub et al. 2011).

[36] The present study was not designed to investigate the specific mechanism(s) of how cells might orient to the vertical gravity vector, though the question of how "gravitaxis" is achieved by microorganisms is intriguing. Physical models for how protists could passively orient vertically due solely to internal weight distribution or asymmetrical cell shape have been proposed (Kessler 1985; Roberts 2006, 2010). These mechanisms could allow negatively buoyant, motile cells to either swim upward or sink slowly depending on the beat patterns of the flagella or cilia, with the reverse occurring for positively buoyant cells. However, such passive models cannot explain the similar swimming speeds between up- and down-swimmers within several species observed here, or observations of *Euglena*, in which gravitaxis was strongly affected by both ultraviolet B radiation (Häder and Liu 1990) and micromolar concentrations of heavy metals (Stallwitz and Häder 1994). The alternative hypothesis is physiological sensing of gravity, which would enable cells to actively orient and rapidly swim either up or down. Indeed,

graviperception has been shown or strongly suggested for a few protists, for example, via statoliths or mechanosensitive ion channels (Hemmersbach and Häder 1999), but the phenomenon is still far from well understood. We suspect graviperception in at least some of the species studied here, particularly those with the most obvious bimodal vertical velocity distributions (i.e., *Oxyrrhis marina*, *Heterocapsa akashiwo*, *Prorocentrum concavum*).

[37] We deliberately suppressed ambient convection currents in these experiments so that swimming behaviors could be isolated. However, it is well known that oceanic turbulence, which is experienced as laminar shear at the scale of individual protists, is an important environmental factor for planktonic organisms (Guasto et al. 2012). In regards to motility patterns, a fundamental, largely unanswered question is the degree to which cell swimming behavior is modified by turbulence-induced shear. Given the lack of experimental data, swimming patterns in the absence of water movement have typically been superimposed onto ambient turbulent velocity fields to yield predicted cell trajectories in flow (e.g., Yamazaki and Kamykowski 1991; Ralston et al. 2007; Ross and Sharples 2008). This practice assumes that swimming behavior is unchanged by turbulence. Work by Kessler et al. (1998) demonstrated a complex vertical reorientation response to rotating shear flow, and another study utilizing similar flow but in the horizontal plane (Karp-Boss et al. 2000) also indicated a significant effect of shear on dinoflagellate swimming patterns but nevertheless suggested that cells could orient effectively under typical turbulent intensities in the field. The passive orientation mechanisms discussed above (e.g., bottom heaviness) can explain certain interactions of swimming cells with turbulence (e.g., observations of patch formation within vortices; Durham et al. 2013), but again, these do not account for the existence of active downward swimming in other situations. A thorough understanding of how swimming microorganisms interact with turbulence will clearly require additional experimental study.

[38] Turbulence is not the only flow feature of the marine environment likely to affect planktonic organisms. Durham et al. (2009) showed that persistent, large-scale horizontal shear can trap gravitactic,

upward-swimming cells, including *Heterocapsa akashiwo*, within thin layers for an extended period of time. Hence, some environmental flow conditions can halt the rapid vertical progress that otherwise ballistic swimmers can make in quiescent conditions. However, persistent swimming behaviors may still enable protists to maintain some control over their position in the face of hydrodynamic forcing. For example, in upwelling regions, dinoflagellates commonly form blooms despite high turbulence, shear, vertical mixing, and advection (Smayda 2010a). Swimming speeds of  $1\text{--}10\text{ m d}^{-1}$ , extrapolated from our observed vertical swimming speeds, are comparable to typical mean upwelling currents of  $0.8\text{--}36\text{ m d}^{-1}$  (Smayda 2000), indicating that these protists could overcome weak to moderate upwelling. Persistent, directed swimming may also allow protists to avoid being flushed out of estuarine habitats by tidal currents (Crawford and Purdie 1992) by transiently seeking refuge at quiescent depths.

### Significance to Aquatic Environments

[39] Technological advances have enabled us to observe tens of thousands of individual protists, representing phylogenetically and nutritionally diverse plankton species, for a longer period of time and within a much less spatially constrained environment than previously possible. The quantitative, 3D data presented in this study indicate a strong vertical bias in the swimming behavior of many protist species, in agreement with many qualitative and a few quantitative reports of predominantly vertical swimming. We observed frequent turning (i.e., very short correlation scales and random motility akin to molecular diffusion) in the horizontal plane but highly correlated, ballistic, straight-line movement in the vertical dimension for the duration of our observations (tens of seconds to minutes). Hence, these cells tended to swim either up or down, rarely changing vertical direction, in the absence of any environmental cues besides gravity. The simultaneous presence of both strongly up- and down-swimming groups suggests active vertical orientation and perception of gravity in several species, though the requisite sensory organelles have yet to be identified.

[40] Our observations of vertically ballistic swimming are consistent with large-scale migratory behaviors



(i.e., Diel vertical migrations, DVM) reported in the field, providing a link between population and individual scales that has rarely been established quantitatively. Whereas most studies of migratory behavior have focused on phytoplankton, we also observed vertically ballistic swimming in two heterotrophic dinoflagellates, but not a ciliate.

[41] Given that vertical gradients in the ocean are generally much sharper than horizontal gradients (e.g., velocity, light, resources), vertically biased motility patterns may provide a considerable adaptive advantage. Rapid ballistic swimming in the vertical direction could allow an organism to exploit these sharp gradients, while undirected diffusive movement in the horizontal plane may be more than sufficient to overcome local resource depletion. However, more experimental work that incorporates ambient fluid shear is needed to discern the degree to which protists maintain such directed ballistic trajectories in the turbulent marine environment.

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